

WHAT IS CLAIMED IS:

1. A method of generating cells capable of secreting insulin, the method comprising:
 - (a) subjecting mammalian embryonic stem cells to a first set of culturing conditions selected suitable for differentiation of at least a portion of said mammalian embryonic stem cells into cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype; and
 - (b) subjecting said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a second set of culturing conditions selected suitable for formation of surface bound cell clusters including insulin producing cells, thereby generating cells capable of secreting insulin.
2. The method of claim 1, further comprising:
 - (c) isolating said surface bound cell clusters and optionally isolating said insulin producing cells therefrom.
3. The method of claim 1, further comprising:
 - (c) dissociating said surface bound cell clusters into single cells including said insulin producing cells; and
 - (d) subjecting said single cells to a third set of culturing conditions selected suitable for maintaining said insulin producing cells in culture for at least 14 days.
4. The method of claim 3, further comprising:
 - (e) isolating said insulin producing cells.
5. The method of claim 3, wherein said third set of culturing conditions is selected suitable for maintaining said insulin producing cells in suspended cell clusters.

6. The method of claim 5, wherein said suspended cell clusters are characterized by a proportion of said insulin producing cells of at least 4 percent.

7. The method of claim 5, wherein an insulin secretion rate capacity of said insulin producing cells of said suspended cell clusters is at least 6 microunits insulin per one hundred thousand cells per hour.

8. The method of claim 5, wherein a total insulin secretion capacity of said insulin producing cells of said suspended cell clusters is at least 0.50 microunits insulin per one hundred thousand cells.

9. The method of claim 5, further comprising:
(e) isolating said suspended cell clusters.

10. The method of claim 3, wherein said third set of culturing conditions is selected suitable for inhibiting growth of substantially non insulin producing cells.

11. The method of claim 10, wherein said substantially non insulin producing cells are neurons and/or mesenchymal cells.

12. The method of claim 3, wherein said dissociating said surface bound cell clusters into single cells is effected by trypsinization of said surface bound cell clusters.

13. The method of claim 3, wherein said third set of culturing conditions includes a condition selected from the group consisting of a substantially serum free culture medium, a basic fibroblast growth factor free culture medium, a culture medium including nicotinamide, a culture medium including a synthetic serum supplement, a culture medium including glucose at a concentration of 15 millimolar or less, and inhibiting adherence of said insulin producing cells to a surface.

14. The method of claim 13, wherein said inhibiting adherence of said insulin producing cells to said surface is effected by culturing said insulin producing

cells on a substantially non cell adherent plastic surface.

15. The method of claim 1, further comprising the step of selectively harvesting said mammalian embryonic stem cells from a culture including feeder cells and said mammalian embryonic stem cells prior to step (a).

16. The method of claim 1, wherein said first set of culturing conditions is selected suitable for inducing formation of embryoid bodies.

17. The method of claim 1, wherein said first set of culturing conditions is selected capable of inhibiting adherence of said mammalian embryonic stem cells to a surface.

18. The method of claim 17, wherein said inhibiting adherence of said mammalian embryonic stem cells to a surface is effected by culturing said mammalian embryonic stem cells on a substantially non cell adherent plastic surface.

19. The method of claim 1, wherein said at least one characteristic associated with a pancreatic islet cell progenitor phenotype is expression and optionally display of nestin.

20. The method of claim 1, further comprising isolating said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype prior to step (b).

21. The method of claim 20, wherein said isolating is effected by subjecting said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a fourth set of culturing conditions selected suitable for inhibiting growth of cells not displaying said at least one characteristic associated with a pancreatic islet cell progenitor phenotype.

22. The method of claim 1, further comprising:

(c) dissociating said cells displaying at least one characteristic associated

with a pancreatic islet phenotype into single cells displaying at least one characteristic associated with a pancreatic islet phenotype; and

- (d) subjecting said single cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a fifth set of culturing conditions selected suitable for proliferation of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype prior to step (b).

23. The method of claim 21, wherein said fourth set of culturing conditions includes a culturing condition selected from the group consisting of a substantially serum free culture medium, a culture medium including insulin, a culture medium including transferrin, a culture medium including fibronectin, a culture medium substantially including selenium, and facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface.

24. The method of claim 23, wherein said facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface is effected by culturing said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype in contact with a tissue culture coated plastic surface.

25. The method of claim 22, wherein said fifth set of culturing conditions includes a condition selected from the group consisting of a substantially serum free culture medium, a culture medium including basic fibroblast growth factor, a culture medium including a synthetic serum supplement, and facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface.

26. The method of claim 25, wherein said facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface is effected by culturing said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype in

contact with a plastic surface coated with gelatin or poly-L-lysine.

27. The method of claim 1, wherein said second set of culturing conditions is selected suitable for formation of cell clusters including cells displaying at least one characteristic associated with a pancreatic islet cell phenotype selected from the group consisting of an endocrine cell precursor phenotype, an alpha cell phenotype, a beta cell phenotype, a delta cell phenotype, and a neuronal cell phenotype.

28. The method of claim 27, wherein said at least one characteristic associated with an endocrine cell precursor phenotype is expression or display of an mRNA of a transcription factor or an mRNA of a glucose transporter.

29. The method of claim 28, wherein said transcription factor is Pax6.

30. The method of claim 28, wherein said glucose transporter is Glut2.

31. The method of claim 27, wherein said at least one characteristic associated with an alpha cell phenotype is expression or display of glucagon mRNA or glucagon.

32. The method of claim 27, wherein said at least one characteristic associated with a beta cell phenotype is selected from the group consisting of expression or display of an mRNA of a transcription factor, an mRNA of a glucose transporter, an mRNA of a glucose metabolism enzyme, and insulin mRNA.

33. The method of claim 32, wherein said transcription factor is selected from the group consisting of Pdx1, Isl1, Beta2, Pax4 and Nkx6.1.

34. The method of claim 32, wherein said glucose transporter is Glut2.

35. The method of claim 32, wherein said glucose metabolism enzyme is glucokinase.

36. The method of claim 27, wherein said at least one characteristic associated with a delta cell phenotype is expression or display of somatostatin.

37. The method of claim 27, wherein said at least one characteristic associated with a neuronal cell phenotype is a neuronal morphology.

38. The method of claim 1, wherein said second set of culturing conditions is selected suitable for formation of cell clusters including insulin producing cells capable of displaying a change in an insulin secretion in response to a drug selected from the group consisting of an increase in said insulin secretion wherein said drug is tolbutamide, an increase in said insulin secretion wherein said drug is IBMX, a decrease in said insulin secretion wherein said drug is diazoxide, a decrease in said insulin secretion wherein said drug is nifedipine, and a decrease in said insulin secretion wherein said drug is carbachol.

39. The method of claim 1, wherein said second set of culturing conditions includes a condition selected from the group consisting of a substantially serum free culture medium, a basic fibroblast growth factor free culture medium, a culture medium including nicotinamide, a culture medium including a synthetic serum supplement, a culture medium including glucose at a concentration of 15 millimolar or less, and facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface.

40. The method of claim 39, wherein said facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface is effected by culturing said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype in contact with a plastic surface coated with gelatin.

41. The method of claim 1, wherein said second set of culturing conditions is selected suitable for formation of cell clusters including insulin producing cells maintainable in culture for at least 7 days.

42. The method of claim 3, wherein said third set of culturing conditions is selected suitable for formation of cell clusters including cells displaying at least one characteristic associated with a pancreatic islet cell phenotype selected from the group consisting of an endocrine cell precursor phenotype, an alpha cell phenotype, a beta cell phenotype, a delta cell phenotype, and a neuronal cell phenotype.

43. The method of claim 42, wherein said at least one characteristic associated with an endocrine cell precursor phenotype is expression or display of an mRNA of a transcription factor or an mRNA of a glucose transporter.

44. The method of claim 43, wherein said transcription factor is Pax6.

45. The method of claim 43, wherein said glucose transporter is Glut2.

46. The method of claim 42, wherein said at least one characteristic associated with an alpha cell phenotype is expression or display of glucagon mRNA or glucagon.

47. The method of claim 42, wherein said at least one characteristic associated with a beta cell phenotype is selected from the group consisting of expression or display of an mRNA of a transcription factor, an mRNA of a glucose transporter, an mRNA of a glucose metabolism enzyme, and insulin mRNA.

48. The method of claim 47, wherein said transcription factor is selected from the group consisting of Pdx1, Isl1, Beta2, Pax4 and Nkx6.1.

49. The method of claim 47, wherein said glucose transporter is Glut2.

50. The method of claim 47, wherein said glucose metabolism enzyme is glucokinase.

51. The method of claim 42, wherein said at least one characteristic associated with a delta cell phenotype is expression or display of somatostatin.

52. The method of claim 42, wherein said at least one characteristic associated with a neuronal cell phenotype is a neuronal morphology.

53. The method of claim 1, wherein said mammalian embryonic stem cells are human embryonic stem cells.

54. The method of claim 53, wherein said human embryonic stem cells are selected from the group consisting of I6 cells, H9 cell derived cells, and H13 cells.

55. The method of claim 54, wherein said H9 cell derived cells are H9.2 cells.

56. An insulin producing cell cluster comprising insulin producing cells being maintainable in culture for at least 14 days, wherein a proportion of said insulin producing cells in the cell cluster is at least 4 percent.

57. The insulin producing cell cluster of claim 56, wherein said proportion of said insulin producing cells in the cell cluster is at least 32 percent.

58. The insulin producing cell cluster of claim 56, wherein an insulin secretion rate capacity of said insulin producing cells is at least 6 microunits insulin per one hundred thousand cells per hour.

59. The insulin producing cell cluster of claim 56, wherein a total insulin secretion capacity of said insulin producing cells is at least 0.50 microunits insulin per one hundred thousand cells.

60. The insulin producing cell cluster of claim 56, wherein the cell cluster further comprises cells displaying at least one characteristic associated with a pancreatic islet cell phenotype selected from the group consisting of an endocrine cell precursor phenotype, an alpha cell phenotype, a beta cell phenotype, a delta cell phenotype, and a neuronal cell phenotype

61. The insulin producing cell cluster of claim 60, wherein said at least one characteristic associated with an endocrine cell precursor phenotype is expression or display of an mRNA of a transcription factor or an mRNA of a glucose transporter.

62. The insulin producing cell cluster of claim 61, wherein said transcription factor is Pax6.

63. The insulin producing cell cluster of claim 61, wherein said glucose transporter is Glut2.

64. The insulin producing cell cluster of claim 60, wherein said at least one characteristic associated with an alpha cell phenotype is expression or display of glucagon mRNA or glucagon.

65. The insulin producing cell cluster of claim 60, wherein said at least one characteristic associated with a beta cell phenotype is selected from the group consisting of expression or display of an mRNA of a transcription factor, an mRNA of a glucose transporter, an mRNA of a glucose metabolism enzyme, and insulin mRNA.

66. The insulin producing cell cluster of claim 65, wherein said transcription factor is selected from the group consisting of Pdx1, Isl1, Beta2, Pax4 and Nkx6.1.

67. The insulin producing cell cluster of claim 65, wherein said glucose transporter is Glut2.

68. The insulin producing cell cluster of claim 65, wherein said glucose metabolism enzyme is glucokinase.

69. The insulin producing cell cluster of claim 60, wherein said at least one characteristic associated with a delta cell phenotype is expression or display of somatostatin.

70. The insulin producing cell cluster of claim 60, wherein said at least one characteristic associated with a neuronal cell phenotype is a neuronal morphology.

71. The insulin producing cell cluster of claim 56, wherein said insulin producing cell cluster produces human insulin.

72. The insulin producing cell cluster of claim 56, wherein said insulin producing cell cluster includes human cells.

73. The insulin producing cell cluster of claim 72, wherein said human cells have a genotype of I6 cells, H9 cell derived cells, and H13 cells.

74. The insulin producing cell cluster of claim 73, wherein said H9 cell derived cells are H9.2 cells.

75. A method of producing insulin, the method comprising:

- (a) subjecting mammalian embryonic stem cells to a first set of culturing conditions selected suitable for differentiation of at least a portion of said mammalian embryonic stem cells into cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype; and
- (b) subjecting said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a second set of culturing conditions selected suitable for formation of surface bound cell clusters including insulin producing cells, thereby producing the insulin.

76. The method of claim 75, further comprising:

- (c) harvesting the insulin.

77. The method of claim 75, further comprising:

- (c) isolating said surface bound cell clusters and optionally isolating said insulin producing cells therefrom.

78. The method of claim 75, further comprising:

- (c) dissociating said surface bound cell clusters into single cells including said insulin producing cells; and
- (d) subjecting said single cells to a third set of culturing conditions selected suitable for maintaining said insulin producing cells in culture for at least 14 days.

79. The method of claim 78, further comprising:

- (e) isolating said insulin producing cells.

80. The method of claim 78, wherein said third set of culturing conditions is selected suitable for maintaining said insulin producing cells in suspended cell clusters.

81. The method of claim 80, wherein said suspended cell clusters are characterized by a proportion of said insulin producing cells of at least 4 percent.

82. The method of claim 80, wherein an insulin secretion rate capacity of said insulin producing cells of said suspended cell clusters is at least 6 microunits insulin per one hundred thousand cells per hour.

83. The method of claim 80, wherein a total insulin secretion capacity of said insulin producing cells of said suspended cell clusters is at least 0.50 microunits insulin per one hundred thousand cells.

84. The method of claim 80, further comprising:

- (e) isolating said suspended cell clusters.

85. The method of claim 78, wherein said third set of culturing conditions is selected suitable for inhibiting growth of substantially non insulin producing cells.

86. The method of claim 85, wherein said substantially non insulin producing cells are neurons and/or mesenchymal cells.

87. The method of claim 78, wherein said dissociating said surface bound cell clusters into single cells is effected by trypsinization of said surface bound cell clusters.

88. The method of claim 78, wherein said third set of culturing conditions includes a condition selected from the group consisting of a substantially serum free culture medium, a basic fibroblast growth factor free culture medium, a culture medium including nicotinamide, a culture medium including a synthetic serum supplement, a culture medium including glucose at a concentration of 15 millimolar or less, and inhibiting adherence of said insulin producing cells to a surface.

89. The method of claim 88, wherein said inhibiting adherence of said insulin producing cells to said surface is effected by culturing said insulin producing cells on a substantially non cell adherent plastic surface.

90. The method of claim 75, further comprising the step of selectively
◦ harvesting said mammalian embryonic stem cells from a culture including feeder cells and said mammalian embryonic stem cells prior to step (a).

91. The method of claim 75, wherein said first set of culturing conditions is selected suitable for inducing formation of embryoid bodies.

92. The method of claim 91, wherein said first set of culturing conditions is selected capable of inhibiting adherence of said mammalian embryonic stem cells to a surface.

93. The method of claim 92, wherein said inhibiting adherence of said mammalian embryonic stem cells to a surface is effected by culturing said mammalian embryonic stem cells on a substantially non cell adherent plastic surface.

94. The method of claim 75, wherein said at least one characteristic associated with a pancreatic islet cell progenitor phenotype is expression and optionally display of nestin.

95. The method of claim 75, further comprising isolating said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype prior to step (b).

96. The method of claim 95, wherein said isolating is effected by subjecting said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a fourth set of culturing conditions selected suitable for inhibiting growth of cells not displaying said at least one characteristic associated with a pancreatic islet cell progenitor phenotype.

97. The method of claim 75, further comprising:

- (c) dissociating said cells displaying at least one characteristic associated with a pancreatic islet phenotype into single cells displaying at least one characteristic associated with a pancreatic islet phenotype; and
- (d) subjecting said single cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a fifth set of culturing conditions selected suitable for proliferation of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype prior to step (b).

98. The method of claim 96, wherein said fourth set of culturing conditions includes a culturing condition selected from the group consisting of a substantially serum free culture medium, a culture medium including insulin, a culture medium including transferrin, a culture medium including fibronectin, a culture medium substantially including selenium, and facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface.

99. The method of claim 98, wherein said facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface is effected by culturing said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype in contact with a tissue culture coated plastic surface.

100. The method of claim 97, wherein said fifth set of culturing conditions includes a condition selected from the group consisting of a substantially serum free culture medium, a culture medium including basic fibroblast growth factor, a culture medium including a synthetic serum supplement, and facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface.

101. The method of claim 100, wherein said facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface is effected by culturing said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype in contact with a plastic surface coated with gelatin or poly-L-lysine.

102. The method of claim 75, wherein said second set of culturing conditions is selected suitable for formation of cell clusters including cells displaying at least one characteristic associated with a pancreatic islet cell phenotype selected from the group consisting of an endocrine cell precursor phenotype, an alpha cell phenotype, a beta cell phenotype, a delta cell phenotype, and a neuronal cell phenotype.

103. The method of claim 102, wherein said at least one characteristic associated with an endocrine cell precursor phenotype is expression or display of an mRNA of a transcription factor or an mRNA of a glucose transporter.

104. The method of claim 103, wherein said transcription factor is Pax6.

105. The method of claim 103, wherein said glucose transporter is Glut2.

106. The method of claim 102, wherein said at least one characteristic associated with an alpha cell phenotype is expression or display of glucagon mRNA or glucagon.

107. The method of claim 102, wherein said at least one characteristic associated with a beta cell phenotype is selected from the group consisting of

expression or display of an mRNA of a transcription factor, an mRNA of a glucose transporter, an mRNA of a glucose metabolism enzyme, and insulin mRNA.

108. The method of claim 107, wherein said transcription factor is selected from the group consisting of Pdx1, Isl1, Beta2, Pax4 and Nkx6.1.

109. The method of claim 107, wherein said glucose transporter is Glut2.

110. The method of claim 107, wherein said glucose metabolism enzyme is glucokinase.

111. The method of claim 102, wherein said at least one characteristic associated with a delta cell phenotype is expression or display of somatostatin.

112. The method of claim 102, wherein said at least one characteristic associated with a neuronal cell phenotype is a neuronal morphology.

113. The method of claim 78, wherein said third set of culturing conditions is selected suitable for formation of cell clusters including cells displaying at least one characteristic associated with a pancreatic islet cell phenotype selected from the group consisting of an endocrine cell precursor phenotype, an alpha cell phenotype, a beta cell phenotype, a delta cell phenotype, and a neuronal cell phenotype.

114. The method of claim 113, wherein said at least one characteristic associated with an endocrine cell precursor phenotype is expression or display of an mRNA of a transcription factor or an mRNA of a glucose transporter.

115. The method of claim 114, wherein said transcription factor is Pax6.

116. The method of claim 114, wherein said glucose transporter is Glut2.

117. The method of claim 113, wherein said at least one characteristic associated with an alpha cell phenotype is expression or display of glucagon mRNA or

glucagon.

118. The method of claim 113, wherein said at least one characteristic associated with a beta cell phenotype is selected from the group consisting of expression or display of an mRNA of a transcription factor, an mRNA of a glucose transporter, an mRNA of a glucose metabolism enzyme, and insulin mRNA.

119. The method of claim 118, wherein said transcription factor is selected from the group consisting of Pdx1, Isl1, Beta2, Pax4 and Nkx6.1.

120. The method of claim 118, wherein said glucose transporter is Glut2.

121. The method of claim 118, wherein said glucose metabolism enzyme is glucokinase.

122. The method of claim 113, wherein said at least one characteristic associated with a delta cell phenotype is expression or display of somatostatin.

123. The method of claim 113, wherein said at least one characteristic associated with a neuronal cell phenotype is a neuronal morphology.

124. The method of claim 1, wherein said second set of culturing conditions is selected suitable for formation of cell clusters including insulin producing cells capable of displaying a change in an insulin secretion in response to a drug selected from the group consisting of an increase in said insulin secretion wherein said drug is tolbutamide, an increase in said insulin secretion wherein said drug is IBMX, a decrease in said insulin secretion wherein said drug is diazoxide, a decrease in said insulin secretion wherein said drug is nifedipine, and a decrease in said insulin secretion wherein said drug is carbachol.

125. The method of claim 75, wherein said second set of culturing conditions includes a condition selected from the group consisting of a substantially serum free culture medium, a basic fibroblast growth factor free culture medium, a culture

medium including nicotinamide, a culture medium including a synthetic serum supplement, a culture medium including glucose at a concentration of 15 millimolar or less, and facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface.

126. The method of claim 125, wherein said facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface is effected by culturing said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype in contact with a plastic surface coated with gelatin.

127. The method of claim 75, wherein said second set of culturing conditions is selected suitable for formation of cell clusters including insulin producing cells maintainable in culture for at least 7 days.

128. The method of claim 75, wherein said mammalian embryonic stem cells are human embryonic stem cells.

129. The method of claim 128, wherein said human embryonic stem cells are selected from the group consisting of I6 cells, H9 cell derived cells, and H13 cells.

130. The method of claim 129, wherein said H9 cell derived cells are H9.2 cells.

131. The method of claim 75, wherein said second set of culturing conditions includes culturing said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype in a culturing medium, and wherein harvesting the insulin is effected by harvesting said culture medium.

132. A method of treating a pancreatic disease in a subject, the method comprising:

- (a) subjecting mammalian embryonic stem cells to a first set of culturing conditions selected suitable for differentiation of at least a portion of

said mammalian embryonic stem cells into cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype;

- (b) subjecting said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a second set of culturing conditions selected suitable for formation of surface bound cell clusters including insulin producing cells; and
- (c) administering a therapeutically effective dose of said insulin producing cells to the subject, thereby treating the pancreatic disease.

133. The method of claim 132, further comprising isolating said surface bound cell clusters and optionally said insulin producing cells therefrom prior to step (c).

134. The method of claim 132, further comprising:

- (d) dissociating said surface bound cell clusters into single cells including said insulin producing cells; and
- (e) subjecting said single cells to a third set of culturing conditions selected suitable for maintaining said insulin producing cells in culture for at least 14 days prior to step (c).

135. The method of claim 134, further comprising isolating said insulin producing cells prior to step (c).

136. The method of claim 134, wherein said third set of culturing conditions is selected suitable for maintaining said insulin producing cells in suspended cell clusters.

137. The method of claim 136, further comprising isolating said suspended cell clusters prior to step (c).

138. The method of claim 136, wherein said suspended cell clusters are characterized by a proportion of said insulin producing cells of at least 4 percent.

139. The method of claim 136, wherein an insulin secretion rate capacity of said insulin producing cells of said suspended cell clusters is at least 6 microunits insulin per one hundred thousand cells per hour.

140. The method of claim 136, wherein a total insulin secretion capacity of said insulin producing cells of said suspended cell clusters is at least 0.50 microunits insulin per one hundred thousand cells.

141. The method of claim 134, wherein said third set of culturing conditions is selected suitable for inhibiting growth of substantially non insulin producing cells.

142. The method of claim 141, wherein said substantially non insulin producing cells are neurons and/or mesenchymal cells.

143. The method of claim 134, wherein said dissociating said surface bound cell clusters into single cells is effected by trypsinization of said surface bound cell clusters.

144. The method of claim 134, wherein said third set of culturing conditions includes a condition selected from the group consisting of a substantially serum free culture medium, a basic fibroblast growth factor free culture medium, a culture medium including nicotinamide, a culture medium including a synthetic serum supplement, a culture medium including glucose at a concentration of 15 millimolar or less, and preventing adherence of said insulin producing cells to a surface.

145. The method of claim 144, wherein said preventing adherence of said insulin producing cells to said surface is effected by culturing said insulin producing cells on a substantially non cell adherent plastic surface.

146. The method of claim 132, further comprising the step of selectively harvesting said mammalian embryonic stem cells from a culture including feeder cells and said mammalian embryonic stem cells prior to step (a).

147. The method of claim 132, wherein said first set of culturing conditions is selected suitable for inducing formation of embryoid bodies.

148. The method of claim 147, wherein said first set of culturing conditions is selected capable of inhibiting adherence of said mammalian embryonic stem cells to a surface.

149. The method of claim 148, wherein said inhibiting adherence of said mammalian embryonic stem cells to a surface is effected by culturing said mammalian embryonic stem cells on a substantially non cell adherent plastic surface.

150. The method of claim 132, wherein said at least one characteristic associated with a pancreatic islet cell progenitor phenotype is expression and optionally display of nestin.

151. The method of claim 132, further comprising isolating said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype prior to step (b).

152. The method of claim 151, wherein said isolating is effected by subjecting said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a fourth set of culturing conditions selected suitable for inhibiting growth of cells not displaying said at least one characteristic associated with a pancreatic islet cell progenitor phenotype.

153. The method of claim 132, further comprising:

- (d) dissociating said cells displaying at least one characteristic associated with a pancreatic islet phenotype into single cells displaying at least one characteristic associated with a pancreatic islet phenotype; and
- (e) subjecting said single cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a fifth set of culturing conditions selected suitable for proliferation of said cells displaying at least one characteristic associated with a pancreatic islet

cell progenitor phenotype prior to step (b).

154. The method of claim 152, wherein said fourth set of culturing conditions includes a culturing condition selected from the group consisting of a substantially serum free culture medium, a culture medium including insulin, a culture medium including transferrin, a culture medium including fibronectin, a culture medium substantially including selenium, and facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface.

155. The method of claim 154, wherein said facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface is effected by culturing said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype in contact with a tissue culture coated plastic surface.

156. The method of claim 153, wherein said fifth set of culturing conditions includes a condition selected from the group consisting of a substantially serum free culture medium, a culture medium including basic fibroblast growth factor, a culture medium including a synthetic serum supplement, and facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface.

157. The method of claim 156, wherein said facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface is effected by culturing said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype in contact with a plastic surface coated with gelatin or poly-L-lysine.

158. The method of claim 132, wherein said second set of culturing conditions is selected suitable for formation of cell clusters including cells displaying at least one characteristic associated with a pancreatic islet cell phenotype selected from the group consisting of an endocrine cell precursor phenotype, an alpha cell

phenotype, a beta cell phenotype, a delta cell phenotype, and a neuronal cell phenotype.

159. The method of claim 158, wherein said at least one characteristic associated with an endocrine cell precursor phenotype is expression or display of an mRNA of a transcription factor or an mRNA of a glucose transporter.

160. The method of claim 159, wherein said transcription factor is Pax6.

161. The method of claim 159, wherein said glucose transporter is Glut2.

162. The method of claim 158, wherein said at least one characteristic associated with an alpha cell phenotype is expression or display of glucagon mRNA or glucagon.

163. The method of claim 158, wherein said at least one characteristic associated with a beta cell phenotype is selected from the group consisting of expression or display of an mRNA of a transcription factor, an mRNA of a glucose transporter, an mRNA of a glucose metabolism enzyme, and insulin mRNA.

164. The method of claim 163, wherein said transcription factor is selected from the group consisting of Pdx1, Isl1, Beta2, Pax4 and Nkx6.1.

165. The method of claim 163, wherein said glucose transporter is Glut2.

166. The method of claim 163, wherein said glucose metabolism enzyme is glucokinase.

167. The method of claim 158, wherein said at least one characteristic associated with a delta cell phenotype is expression or display of somatostatin.

168. The method of claim 158, wherein said at least one characteristic associated with a neuronal cell phenotype is a neuronal morphology.

169. The method of claim 1, wherein said second set of culturing conditions is selected suitable for formation of cell clusters including insulin producing cells capable of displaying a change in an insulin secretion in response to a drug selected from the group consisting of an increase in said insulin secretion wherein said drug is tolbutamide, an increase in said insulin secretion wherein said drug is IBMX, a decrease in said insulin secretion wherein said drug is diazoxide, a decrease in said insulin secretion wherein said drug is nifedipine, and a decrease in said insulin secretion wherein said drug is carbachol.

170. The method of claim 132, wherein said second set of culturing conditions includes a condition selected from the group consisting of a substantially serum free culture medium, a basic fibroblast growth factor free culture medium, a culture medium including nicotinamide, a culture medium including a synthetic serum supplement, a culture medium including glucose at a concentration of 15 millimolar or less, and facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface.

171. The method of claim 170, wherein said facilitating adherence of said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype to a surface is effected by culturing said cells displaying at least one characteristic associated with a pancreatic islet cell progenitor phenotype in contact with a plastic surface coated with gelatin.

172. The method of claim 132, wherein said second set of culturing conditions is selected suitable for formation of cell clusters including insulin producing cells maintainable in culture for at least 7 days.

173. The method of claim 134, wherein said third set of culturing conditions is selected suitable for formation of cell clusters including cells displaying at least one characteristic associated with a pancreatic islet cell phenotype selected from the group consisting of an endocrine cell precursor phenotype, an alpha cell phenotype, a beta cell phenotype, a delta cell phenotype, and a neuronal cell phenotype.

174. The method of claim 173, wherein said at least one characteristic associated with an endocrine cell precursor phenotype is expression or display of an mRNA of a transcription factor or an mRNA of a glucose transporter.

175. The method of claim 174, wherein said transcription factor is Pax6.

176. The method of claim 174, wherein said glucose transporter is Glut2.

177. The method of claim 173, wherein said at least one characteristic associated with an alpha cell phenotype is expression or display of glucagon mRNA or glucagon.

178. The method of claim 173, wherein said at least one characteristic associated with a beta cell phenotype is selected from the group consisting of expression or display of an mRNA of a transcription factor, an mRNA of a glucose transporter, an mRNA of a glucose metabolism enzyme, and insulin mRNA.

179. The method of claim 178, wherein said transcription factor is selected from the group consisting of Pdx1, Isl1, Beta2, Pax4 and Nkx6.1.

180. The method of claim 178, wherein said glucose transporter is Glut2.

181. The method of claim 178, wherein said glucose metabolism enzyme is glucokinase.

182. The method of claim 173, wherein said at least one characteristic associated with a delta cell phenotype is expression or display of somatostatin.

183. The method of claim 173, wherein said at least one characteristic associated with a neuronal cell phenotype is a neuronal morphology.

184. The method of claim 132, wherein said mammalian embryonic stem cells are human embryonic stem cells.

185. The method of claim 184, wherein said human embryonic stem cells are selected from the group consisting of I6 cells, H9 cell derived cells, and H13 cells.

186. The method of claim 185, wherein said H9 cell derived cells are H9.2 cells.

187. The method of claim 132, wherein said insulin producing cells are syngeneic with or allogeneic with the subject.

188. The method of claim 132, wherein the subject is a human or a non human mammal.

189. The method of claim 133, wherein step (c) is effected by administering said isolated surface bound cell clusters to the subject.

190. The method of claim 136, wherein step (c) is effected by administering said suspended cell clusters to the subject.

191. The method of claim 137, wherein step (c) is effected by administering said isolated suspended cell clusters to the subject.

192. The method of claim 132, wherein said administering is effected by transplantation or injection of said insulin producing cells into the pancreas of the subject.